The Taxa of *Rhymogona* (Diplopoda: Craspedosomatidae): a Ring Species Part One: Genetic Analysis of Population Structure

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ABSTRACT

The genetic analysis of the population structure of *Rhymogona* is based on allozyme data from vertical starch gel electrophoresis (14 enzyme loci surveyed) and includes all taxa (73 collecting sites). The genetic structure of *Rhymogona* populations is not consistent with current taxonomy. We find five major groups of populations which are arranged in a circular fashion around the Jura. Adjacent groups differ from each other in allele substitutions at five polymorphic loci altogether and are connected by clinal variation. The extreme populations of this ring differ in allele substitutions at four loci. They are found in Switzerland where they obviously came into secondary contact after the last glaciation. They form narrow hybrid zones in the Jura and the Alps. Our data suggest that *Rhymogona* must be regarded as a polytypic species if the biological species concept is applied.

RÉSUMÉ

***Rhymogona* (Diplopoda, Craspedosomatidae), un genre monospécifique. Première partie : analyse génétique de la structure des populations.**

L'analyse génétique de la structure des populations de *Rhymogona* est basée sur les observations des allozymes par électrophorèse sur gel d'amidon vertical (14 loci enzymatiques) et porte sur tous les taxons de ce genre récoltés dans 73 stations. La structure génétique des populations de *Rhymogona* ne coïncide pas avec la taxinomie usuelle. Nous constatons qu'il existe cinq principaux groupes de populations, celles-ci étant distribuées de manière circulaire autour du Jura. Les groupes adjacents se distinguent par des substitutions alléliques dans cinq loci polymorphiques et sont reliés par des variations clinales. Les populations se situant aux extrêmes se distinguent par des substitutions alléliques dans quatre loci. Elles ont été recensées en Suisse où, de toute évidence, elles semblent être entrées secondairement en contact après la dernière glaciation. Elles forment d'étroites zones hybrides dans le Jura et les Alpes. Sur la base de nos résultats, *Rhymogona* doit être considéré comme espèce polytypique si l'on veut tenir compte du concept biologique de l'espèce.

SCHOLL, A. & PEDROLI-CHRISTEN, A., 1996. — The taxa of *Rhymogona* (Diplopoda: Craspedosomatidae): a ring species. Part one: genetic analysis of population structure. *In*: GEOFFROY, J.-J., MAURIÈS, J.-P. & NGUYEN DUY - JACQUEMIN, M., (eds), Acta Myriapodologica. *Mém. Mus. natn. Hist. nat.*, **169** : 45-51. Paris ISBN : 2-85653-502-X.

INTRODUCTION

Rhymogona is a small genus of the Diplopod family Craspedosomatidae which lives north of the Swiss Alps and in adjacent parts of France and Germany. Seven nominal species are recognized, most of these are known from one or a few localities only (PEDROLI-CHRISTEN & SCHOLL, this volume). We have initially attempted to study the distribution in detail (PEDROLI-CHRISTEN, 1990). Since species identification in this genus is based essentially on subtle differences in morphology of genitalia and is often not unambiguous, we have asked if enzyme electrophoretic data might be used as additional information for species identification. Our samples cover the whole area of distribution of *Rhymogona* and all taxa described (73 collecting sites).

MATERIAL AND METHODS

The collecting sites are shown in Fig. 1 and are listed in Table 1 along with the species diagnosis based on morphological criteria (PEDROLI-CHRISTEN & SCHOLL, this volume, for details of taxonomy and identification). The specimens were stored at -80°C prior to electrophoresis. Electrophoretic studies (vertical starch gel electrophoresis) were conducted using routine techniques of our laboratory (cf. SCHOLL *et al.*, 1990; PEDROLI-CHRISTEN & SCHOLL, 1990). We have scored 14 loci: Apk, Got-1, Got-2, α-Gpd, Gpt, Hk, Idh, Mdh-1, Mdh-2, Mod, Mpi, 6Pgd, Pgi and Pk. Five loci were polymorphic and indicated genetic differentiation among populations: Got-1, Mpi, 6Pgd, Pgi and Pk.

Mendelian inheritance of the electromorphs observed could not be assessed by breeding experiments but is assumed by analogy (cf. ZIMMERMANN & SCHOLL, 1993). Due to initial difficulties in resolving Mpi, this enzyme was not scored in some populations (Table 1). The designation of alleles is based on electrophoretic mobilities (in mm) of the electromorphs; *R. montivaga* from the Alps (sites 2-5 in Table1 and Fig. 1) were used as reference (assigned index = 100 for the common allele at each locus). Coefficients of genetic identity (I) were calculated in pairwise comparisons of the populations using the formula given by NEI (1972). These coefficients served as a matrix for average linkage cluster analysis (UPGMA) (NEI, 1987).

RESULTS AND DISCUSSION

Table 1 shows the allele frequencies at five polymorphic loci. We have not listed seven very rare alleles which were observed at one or the other locus in nine populations altogether. These alleles do not contribute to the outline of the *Rhymogona* population structure presented. Sample sizes were very low in some collecting sites. We have listed these sites in Table 1, but samples with < 5 specimens were not included in further treatment of data because adequate sample sizes are critical in genetic analysis of populations. Identification of individuals based on morphology suggested that several samples contained more than one taxon (e.g. sites 27, 33, 53 in Table 1 and Fig. 1). The electrophoretic data, however, gave no evidence for the coexistence of genetically separated gene pools at these sites or at any other site. With respect to the enzyme phenotypes observed, we found no significant deviations from HARDY-WEINBERG expectations. For calculations of allele frequencies and further treatment of data we have pooled all specimens of a particular site.

Two alleles were found at the Got-1 locus, Got-1^{100} and Got-1^{96} respectively. In most populations, however, one or the other allele was fixed. Allele Got-1^{100} was observed in most *R. montivaga* populations (sites 1 - 11 in Table 1), *montivaga* populations from the western part of the Swiss Jura (sites 12 - 14) were polymorphic, French *R. montivaga* populations (sites 15 - 16) instead had allele Got-1^{96} fixed as all other populations and taxa except *montivaga/cervina* hybrid populations (sites 67 - 73).

Two alleles were found at the 6-Pgd locus, 6-Pgd^{100} and 6-Pgd^{94} respectively. Allele 6-Pgd^{100} was fixed in all populations and taxa except the Swiss *R. cervina* populations. The *R. cervina* populations in the Swiss Jura (sites 55 - 62) had allele 6-Pgd^{94} fixed; *R. cervina* populations along the Rhine (sites 44 - 47, 50 and 53), populations from the *cervina/alemannica* contact zone in the Swiss Jura (sites 26, 27) and populations from the *montivaga/cervina* hybrid zone in the Swiss Jura and the Alps (sites 65 - 73) were polymorphic.

	Sampling Site	Taxon (based on morphology)	number of specimens	Allele Frequencies Got-1 96	Got-1 100	6Pgd 94	6Pgd 100	Pk 94	Pk 100	Pgi 100	Pgi 103	Mpi 100	Mpi 102	Mpi no activity
1	Leukerbad	*m. montivaga*	4		1.00		1.00		1.00	1.00		-		
2	Gemmi	*m. montivaga*	16	0.16	0.84		1.00		1.00	1.00		0.37		0.63
3	Iffigenalp	*m. montivaga*	13		1.00		1.00		1.00	0.96		0.96	0.04	
4	Sanetsch	*m. montivaga*	12		1.00	0.04	0.96		1.00	1.00		1.00		
5	Lauenen	*m. montivaga*	13		1.00		0.96		1.00	1.00		1.00		
6	Tour de Famelon	*m. montivaga*	7		1.00		1.00		1.00	1.00		1.00		
7	Morgins	*m. montivaga*	4		1.00		1.00		1.00	1.00		1.00		
8	Vouvry	*m. montivaga*	1		1.00		1.00		1.00	1.00		-		
9	Rochers de Naye	*m. montivaga*	1		1.00		1.00		1.00	1.00		-		
10	Lessy	*m. montivaga*	1		1.00		1.00		1.00	-		-		
11	Le Cachot	*m. montivaga*	1		1.00		1.00		1.00	1.00		-		
12	La Brévine	*m. montivaga*	19	0.05	0.95		1.00		1.00	1.00		1.00		
13	Mauborget	*m. montivaga*	64	0.10	0.90		1.00		1.00	1.00		0.89	0.12	
14	St. Georges	*m. montivaga*	39	0.19	0.81		1.00		1.00	1.00		1.00		
15	Grande Chartreuse	*m. montivaga*	10	1.00			1.00		1.00	1.00		1.00		
16	Levier	*m. montivaga*	11	1.00			1.00		1.00	1.00		0.96	0.04	
17	Deschaux	*m. hessei*	4	1.00			1.00		1.00	1.00		1.00		
18	Médière	*m. hessei*	8	1.00			1.00		1.00	1.00		0.90	0.10	
19	Belverne	*m. hessei*	7	1.00			1.00		1.00	1.00		0.21	0.79	
20	Beaune	*m. hessei*	1	1.00			1.00		1.00	1.00			1.00	
21	Ancey	*montivaga-group**	1	1.00			1.00		1.00	1.00			1.00	
22	Lantenay	*montivaga-group**	1	1.00			1.00		1.00	1.00			1.00	
23	Vernot	*m. hessei*	10	1.00			1.00		1.00	1.00			1.00	
24	Courtedoux	*alemannica*	46	1.00			1.00	0.81	0.19	1.00			0.96	
25	Noir Bois	*alemannica*	28	1.00			1.00	0.86	0.14	1.00			1.00	
26	Le Breuil	*cervina, alemannica***	3	1.00		0.83	0.17	0.83	0.17	1.00			1.00	
27	St. Ursanne	*cervina, alemannica***	31	1.00		0.38	0.63	0.96	0.04	0.98			1.00	
28	Bonfol	*alemannica*	1	1.00			1.00	1.00		1.00			1.00	
29	Boncourt	*alemannica ?*	4	1.00			1.00	0.50	0.50	1.00			1.00	
30	Masevaux	*alemannica*	5	1.00			1.00	1.00		1.00			1.00	
31	Le Haut-du-Them	*alemannica*	2	1.00			1.00	1.00		1.00			1.00	
32	Linthal	*alemannica*	8	1.00			1.00	1.00		1.00			1.00	
33	Kenzingen	*cervina + alemannica ?*	5	1.00			1.00	1.00		1.00			1.00	
34	Hornberg	*cervina, verhoeffi***	3	1.00			1.00	1.00		1.00			1.00	
35	Marbach	*cervina-group**	2	1.00			1.00	1.00		1.00			1.00	
36	Badenweiler	*alemannica*	25	1.00			1.00	0.88	0.10	1.00			1.00	
37	Ottwangen	*serrata*	23	0.91	0.07		1.00	0.39	0.61	1.00			0.98	
38	Inzlingen	*serrata*	10	1.00			1.00	0.20	0.80	0.85	0.15		1.00	
39	Hasel	*wehrana*	26	1.00			1.00	1.00		0.98	0.02		1.00	
40	Todtmoos	*wehrana*	20	1.00			1.00	0.95	0.05	0.95			1.00	
41	Menzenschwand	*wehrana*	3	1.00			1.00	1.00		1.00			-	
42	Laufenburg	*verhoeffi*	5	1.00			1.00	1.00			1.00		0.37	0.63
43	Tiefenstein	*verhoeffi*	5	1.00			1.00	1.00		0.10	0.90		1.00	
44	Sulz	*cervina-group**	7	1.00		0.29	0.71	1.00		0.86	0.14		0.93	
45	Schupfart	*cervina-group**	4	1.00		0.50	0.50	1.00		1.00			0.50	0.50
46	Homburg	*cervina*	16	1.00		0.32	0.68	1.00		0.97	0.03		1.00	
47	Küssnach	*cervina*	4	1.00		0.13	0.88	1.00		1.00			0.50	
48	Altglashütten	*cervina + verhoeffi ?*	4	1.00			1.00	1.00		1.00			1.00	
49	Gutachbrücke	*verhoeffi, wehrana***	4	1.00			1.00	0.50	0.50	1.00			0.75	
50	Husmersee	*cervina*	11	1.00		0.70	0.30	1.00		1.00			1.00	
51	Dissenhofen	*cervina*	2	1.00			1.00	1.00		1.00				1.00
52	Hemishofen	*cervina*	14	1.00			1.00	1.00		1.00				1.00
53	Staad	*cervina, alemannica***	8	1.00		0.13	0.88	1.00		1.00			1.00	
54	Baar	*cervina*	36	1.00		0.95	0.05	0.99		1.00			0.02	0.98
55	Obergütsch	*cervina ?*	2	1.00		1.00		0.75	0.25	1.00			1.00	
56	Trub	*cervina*	7	1.00		0.86	0.14	1.00		1.00				1.00
57	Schelten	*cervina*	4	1.00		1.00		1.00		1.00			-	
58	Berlincourt	*cervina*	20	1.00		1.00		1.00		1.00			0.61	0.39
59	Gorges de Court	*cervina*	3	1.00		1.00		0.33	0.67	1.00			1.00	
60	Combe Biosse	*cervina*	5	1.00		1.00		1.00		1.00				1.00
61	Pertuis	*cervina*	20	1.00		1.00		1.00		1.00			1.00	
62	Prévoux	*cervina*	52	0.99	0.01	0.97	0.03	0.76	0.21	1.00			0.65	0.35
63	Valsainte	*cervina-group**	1	1.00			1.00	1.00		1.00				1.00
64	Schwarzenmatt	*cervina*	3	1.00		1.00		0.33	0.67	1.00			0.18	0.82
65	Jaun	*cervina-group**	3	1.00		0.83	0.17	1.00		1.00				1.00
66	Kandersteg	*cervina*	20	0.95	0.05	0.95	0.05	0.58	0.43	1.00		0.08		0.92
67	Zweisimmen	*cervina / montivaga*	20	0.48	0.53	0.15	0.85	0.25	0.75	1.00		0.28	0.42	0.30
68	Boltigen	*cervina / montivaga*	25	0.78	0.22	0.75	0.25	0.71	0.30	1.00		0.15		0.85
69	Peseux	*cervina / montivaga*	27	0.43	0.57	0.71	0.29	0.06	0.94	1.00		1.00		
70	Rochefort	*cervina / montivaga*	3	0.67	0.33	0.83	0.17	-	-	-				
71	Mauvaise Combe	*cervina / montivaga*	12	0.67	0.33	0.46	0.54	0.13	0.88	1.00		-		
72	La Chaux-du-Milieu	*cervina / montivaga*	39	0.67	0.33	0.60	0.40	0.18	0.82	1.00		-		
73	Verrières	*cervina / montivaga*	38	0.84	0.16	0.57	0.43	0.03	0.93	1.00		0.47	0.05	0.48

TABLE 1. — Allele frequencies at five polymorphic loci (rare alleles are not listed). * = females only; ** = identification ambiguous; sites 67-73 = *cervina/montivaga* hybrid populations.

Two alleles, Pk100 and Pk94 respectively, were found at the Pk locus. Allele Pk100 was fixed in all *R. montivaga* populations. Polymorphism was observed in *R. alemannica* populations from the Swiss Jura (sites 24 - 29), in populations from the southwestern Black Forest region (sites 36 - 40) which were keyed out as *alemannica*, *serrata* and *wehrana*, and in the *montivaga /cervina* hybrid zone.

FIG. 1. — Sampling sites of electrophoretically analysed *Rhymogona* specimens (species diagnosis based on morphological criteria).

At the Pgi locus most populations were monomorphic for allele Pgi100. A second allele, Pgi103, was observed in low frequencies or even fixed in six populations from the southern Black Forest region, including the taxa *serrata*, *wehrana*, *verhoeffi* and *cervina*, as shown in Table 1.

Due to initial difficulties in resolving Mpi, this enzyme was not scored in all populations. Furthermore, many specimens, in particular those from *R. cervina* populations in Switzerland and those from *montivaga/cervina* hybrid populations, failed to show Mpi activity. Possibly this is due to the presence of a null allele. For calculation of Mpi allele frequencies we have assumed that specimens with no Mpi activity are homozygous for a null allele.

In populations of *R. montivaga* and in *montivaga/cervina* hybrid populations we scored the allele Mpi100. Populations of other taxa were usually monomorphic for Mpi102, except two populations from the French Jura (sites 18 and 19), both keyed out as *R. m. hessei*, which were polymorphic. The allele frequencies observed in these two populations suggested clinal variation towards populations from the Vosges.

Cluster analysis of coefficients of genetic identity (I) (populations from the *montivaga/cervina* hybrid zone, sites 66 - 73, not included) resulted in several major groups of populations with very high levels of genetic identity (I > 0.98). These groups are shown in Fig. 2. Group A has the Swiss *R. montivaga* populations; group B has French populations keyed out as *R. m. montivaga* and *R. m. hessei* respectively; group C has *m. hessei* populations; group D has the northern *Rhymogona* populations and includes the taxa *alemannica, cervina*, and *wehrana*; groups E and F have *R. serrata* and *R. verhoeffi*, respectively; group G has the Swiss *R. cervina* populations and includes specimens from the type locality of *R. aelleni* (site 54). These groups usually differ, in the order as they are presented, by allele substitution at one locus (Fig. 2). Group E which has the two *R. serrata* populations is exceptional because it is polymorphic at the Pk locus and therefore has an intermediate position between groups C and D. According to the allele frequencies observed (Table 1) group E is more close to group C with respect to genetic identity.

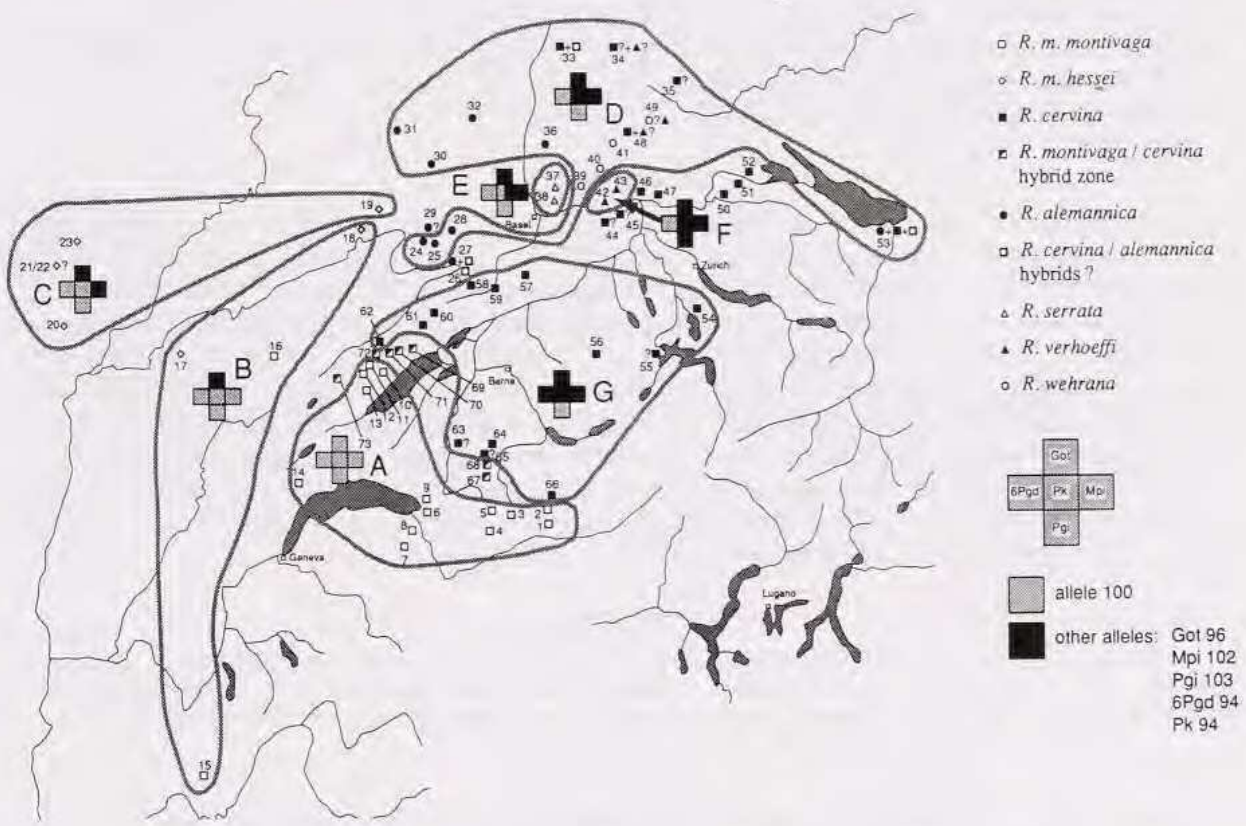

FIG. 2. — Genetic differentiation of *Rhymogona* populations.

It is important, however, to realize that the differentiation among these groups is not abrupt. The allele substitutions observed between population groups change in a clinal fashion. These clines appear to be shallow in some regions and steeper in other regions, as far as we can see from a rather limited number of individuals and/or populations in some areas.

More generally, the electrophoretic data show that the genetic structure of *Rhymogona* populations is not consistent with current taxonomy. This is most clearly evident from a comparison of populations from the Black Forest region and from the Vosges (group D in Fig. 2), which include the taxa *alemannica*, *cervina* and *wehrana* according to morphology. These populations are largely identical with respect to the alleles observed and to their frequencies (Table 1). In contrast, *R. cervina* populations from Switzerland are different from *R. cervina* populations in the Black Forest region. Furthermore, *R. verhoeffi*, which has the allele Mpi^{103} substituted for Mpi^{100}, is clearly differentiated from the other taxa, however, Mpi^{103} is also observed in low frequencies in other populations from nearby localities (sites 38, 39, 44 and 46 in Table 1 and Fig. 1). These specimens were keyed out as *serrata*, *wehrana*, and *cervina* respectively. The electrophoretic data suggest gene flow among these taxa and an isolation-by-distance model of genetic differentiation.

The more relevant information obtained from the electrophoretic survey are the observations that the alleles and their frequencies change largely independently of morphological characters and that they change in a clinal fashion within and among taxa. The groups of populations are arranged in a more or less circular fashion around the Jura. Groups A and G, which have obviously colonized this area after the last glaciation, come into secondary contact in the Swiss Jura and in the Alps. These two groups differ by allele substitution in four loci, and they form rather narrow hybrid zones in the Swiss Jura and the Alps, as we have shown previously (PEDROLI-CHRISTEN & SCHOLL, 1990).

CONCLUSIONS

Rhymogona species, as in most other diplopods, were initially described using the morphospecies concept. Other species concepts have been developed since (cf. HAFFNER, 1986). The biological species concept which defines species as "groups of interbreeding natural populations that are reproductively isolated from other such groups" (MAYR, 1969) is now chosen by the majority of zoologists (MAYR, 1963, 1970; HEWITT, 1990). As summarized in Figure 2, our data show that *Rhymogona* consists of groups of genetically differentiated populations. However, there is no evidence that these groups are reproductively isolated. In contrast, our data show that there is gene flow between these groups. Our results therefore have taxonomic consequences and suggest that *Rhymogona* must be regarded as a polytypic species. All species presently recognized should be revised to subspecies of *Rhymogona montivaga* as will be discussed (PEDROLI-CHRISTEN & SCHOLL, this volume).

ACKNOWLEDGEMENTS

We are indepted to Mrs. V. SIEGFRIED and Mrs. L. FRAUCHIGER for technical assistance in the electrophoretic studies and to S. HUNZIKER for computer graphics. Critical comments and suggestions of Dr. Henrik ENGHOFF and Dr. Adam H. PORTER and Dr. John R. SPENCE on an earlier version of this manuscript are gratefully acknowledged.

REFERENCES

HAFFNER, J., 1986. — Superspecies and species limits in vertebrates. *Z. zool. Syst. Evolutionsforsch.*, **24** : 169-190.

HEWITT, G. M., 1990. — Divergence and speciation as viewed from an insect hybrid zone. *Can. J. Zool.*, **68** : 1701-1715.

MAYR, E., 1963. — *Animal species and evolution*. Cambridge, Massachusetts, Harvard University Press.

MAYR, E., 1969. — *Principles of systematic zoology*. New York, McGraw-Hill.

MAYR, E., 1970. — *Populations, species and evolution*. Cambridge, Massachusetts, Belknap Press of Harvard University.

NEI, M., 1972. — Genetic distance between populations. *Am. Nat.* **106** : 283-292.

NEI, M., 1987. — *Molecular evolutionary genetics*. New-York, 512 pp.

PEDROLI-CHRISTEN, A., 1990. — Field investigations on *Rhymogona cervina* Verhoeff and *Rhymogona silvatica* Rothenbühler (Diplopoda): Morphology, distribution and hybridization. *In* : A. MINELLI, *Proc. 7th int. Congr. Myriapodology*. Leiden, E. J. Brill : 27-43.

PEDROLI-CHRISTEN, A. & SCHOLL, A., 1990. — Ecological and genetic studies on parapatric *Rhymogona silvatica* Roth. and *R. cervina* Verh. (Diplopoda: Craspedosomatidae) with special reference to hybrid populations in a zone of contact. *Rev. suisse Zool.*, **97**, 349-359.

SCHOLL, A., OBRECHT, E. & OWEN, R. E., 1990. — The genetic relationship between *Bombus moderatus* Cresson and the *Bombus lucorum* auct. species complex (Hymenoptera: Apidae). *Can. J. Zool.*, **68** : 2264-2268.

ZIMMERMANN, M. & SCHOLL, A., 1993. — Specific status of *Aquarius cinereus* (Puton) and *Aquarius najas* (De Geer) (Hemiptera: Gerridae) and the extent of hybridisation in the Mediterranean region. *Ent. scand.*, **24** : 197-210.